CHROMOSOMES OF TWO SPECIES OF QUAHOG CLAMS AND THEIR HYBRIDS

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Two species of quahogs (clams of the genus *Mercenaria*, formerly *Venus*) occur along the Atlantic and Gulf coasts of North America. Abbott (1954) characterizes the two species as follows: The northern quahog, *Mercenaria mercenaria* (L.), ranges from the Gulf of St. Lawrence to Florida and the Gulf of Mexico. It has a characteristic smoothish or glossy area on the exterior center of the valves. The interior of the valves is white and commonly has purple stainings. The entire lunule is three-fourths as wide as long. Two subspecies are listed: *M. m. notata* Say with external zigzag brown mottlings and *M. m. texana* Dall, from the northern Gulf of Mexico, with large irregular coalescing flat-topped concentric ribs. The southern quahog, *M. campechiensis* (Gmelin), ranges from the Chesapeake Bay to Florida, Texas, and Cuba. It has a more obese shell and lacks the smooth central area on the exterior of the shells. The entire lunule is usually as wide as long. Rarely are there brown mottlings on the exterior of the valves, which are always white internally.

It is often difficult to assign a specimen to either species if a single character is considered. Fast-growing specimens of *M. mercenaria*, less than about 25 mm. long, lack the characteristic glossy smooth area on the exterior of the valves. Measurements of length and weight of the two species, grown under the same conditions, have shown the small *M. mercenaria* to have heavier shells than *M. campechiensis* of the same length. Otherwise typical individuals of *M. campechiensis* occur with internal purple shell stainings and with the brown mottlings of the subspecies *M. m. notata*. Often the lunule of *M. campechiensis* is only three-fourths as wide as long.

The two species hybridize readily in the laboratory (Loosanoff, 1954). This paper reports chromosome numbers and behavior in the two species and their hybrids at meiosis and early embryonic mitoses.

Materials and Methods

Live specimens of *M. mercenaria* were secured from Connecticut, New York, Delaware, Virginia, North Carolina, South Carolina and the east coast of Florida. The southern quahog was obtained from North Carolina, the east coast of Florida and several localities along the Gulf coast of Florida from Tampa Bay northward. These clams have been used in several ways: for growth experiments (Menzel, 1961a, 1962); for clam farming observations (Menzel, 1961b; Menzel and Sims, 1962); and as brood clams for observations on hybrids (Menzel, 1964). In addi-

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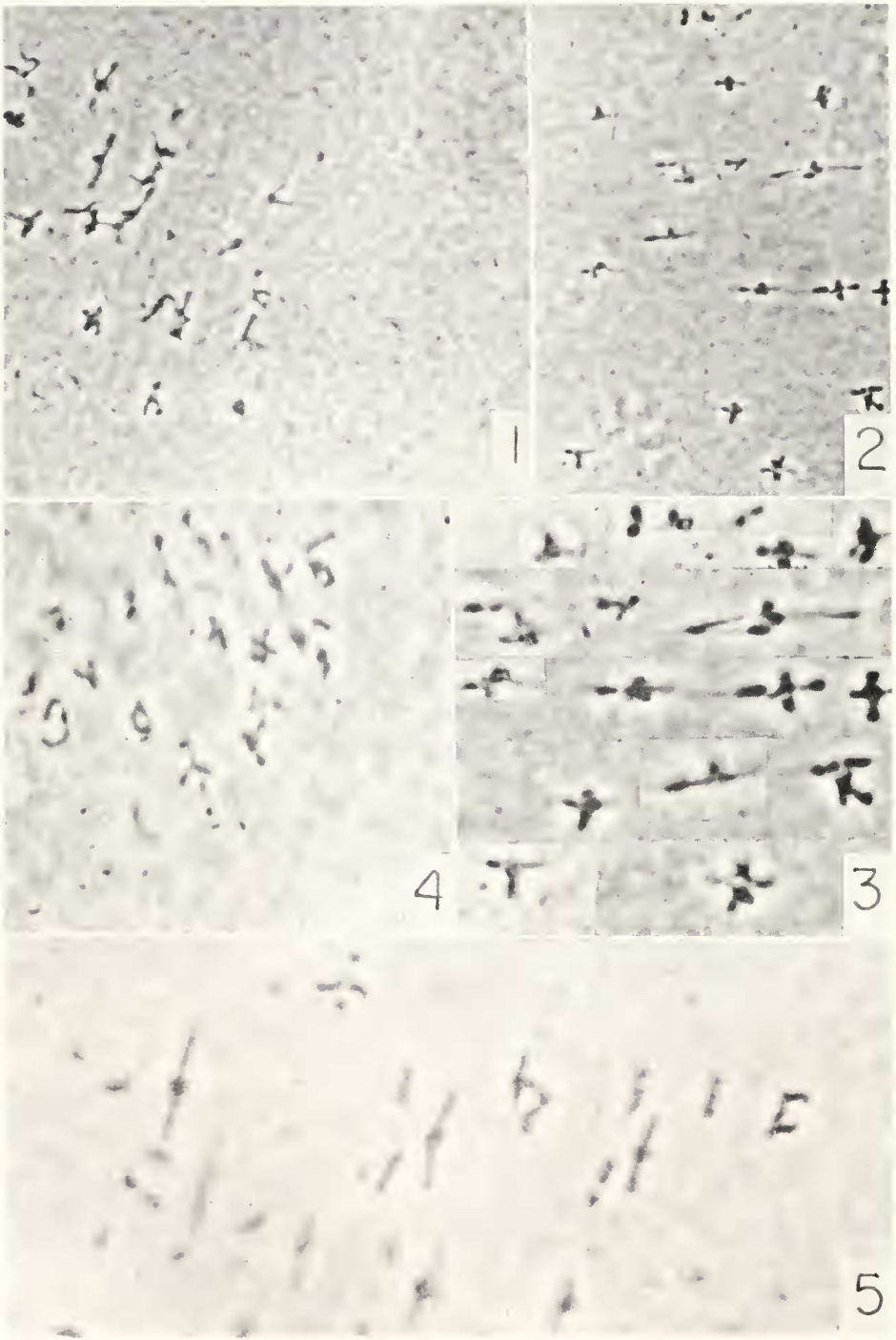


FIGURES 1–5.

tion, laboratory-reared hybrids have been available from the Biological Laboratory, Bureau of Commercial Fisheries, Milford, Connecticut, and from the marine laboratory of the Oceanographic Institute.

Crosses have been made in our laboratory using as brood clams hybrids grown to sexual maturity here and the two species from localities listed above. These crosses include intraspecific crosses, reciprocal crosses of the two species (F_1's), F_2's of the hybrids ♀ *M. campechiensis* × ♂ *M. mercenaria* and reciprocal backcrosses of the latter F_1 to each species. All of the above combinations have been spawned and reared beyond metamorphosis and settling by the techniques of Loosanoff and Davis (1963). Observations of meiosis in eggs from F_1 hybrids reported here were all made on hybrids from crosses between *M. mercenaria* males from Connecticut and *M. campechiensis* females from Florida.

At intervals after spawning, the eggs and embryos were fixed in freshly mixed acetic alcohol (three parts absolute ethanol, one part glacial acetic acid) and stored in a freezer at −16° to −18° C. Several dozen embryos in a small drop of fixative were placed on a slide and air-dried or flamed. Several drops of iron-acetocarmine were added and allowed to stain for two minutes. A coverslip was added and excess stain removed by blotting. The coverslip was pressed firmly on the slide and the preparation was then heated judiciously over an alcohol flame to clear the cytoplasm and further flatten the eggs. Such temporary squashes usually were examined at once with a Zeiss microscope equipped with an apochromatic optical system and phase contrast accessories, but they could be stored for several days at 2°–4° C. without severe deterioration.

Chromosomes prepared in this way were usually well spread but rather lightly stained. Substitution of aceto-orcein, propio-orcein, Gomori's chrom-alum-hematoxylin and Feulgen staining did not result in significantly better preparations. Phase-contrast illumination of the acetocarmine slides was used routinely to enhance contrast and facilitate analysis. Stages of meiosis from metaphase I to telophase II and mitotic figures from early cleavage divisions were readily observed by this method. Because of the dense cytoplasm and tough egg membranes, the eggs were difficult to flatten sufficiently for microphotography. Hence, most of the stages described here are illustrated with drawings made with the aid of a camera lucida.

Preparations from *M. campechiensis* were consistently better than those from the F_1 hybrid, which were in turn better than those from *M. mercenaria*.

Observations

Early embryology

Eggs fixed from 15 seconds to 5 minutes after spawning contained oocyte nuclei at metaphase I regardless of whether sperm suspension had been added. If the eggs were not fertilized, the oocyte nuclei remained at metaphase I for 60 minutes or longer and then gradually degenerated *in situ*. In one unfertilized lot of eggs,

Figures 1–5. Photomicrographs of meiotic metaphase I in clam eggs, phase-contrast illumination. Some of the bivalents are out of focus in each photograph.

Figure 1. *Mercenaria campechiensis*, same nucleus as Figure 8, × 900.

Figure 2. *M. campechiensis*, same nucleus as Figure 10, × 900.

Figure 3. Individual bivalents of nucleus shown in Figures 2 and 10, × 1800.

Figure 4. F_1 hybrid, × 900.

Figure 5. *M. mercenaria*, same nucleus as Figure 12, × 1800.

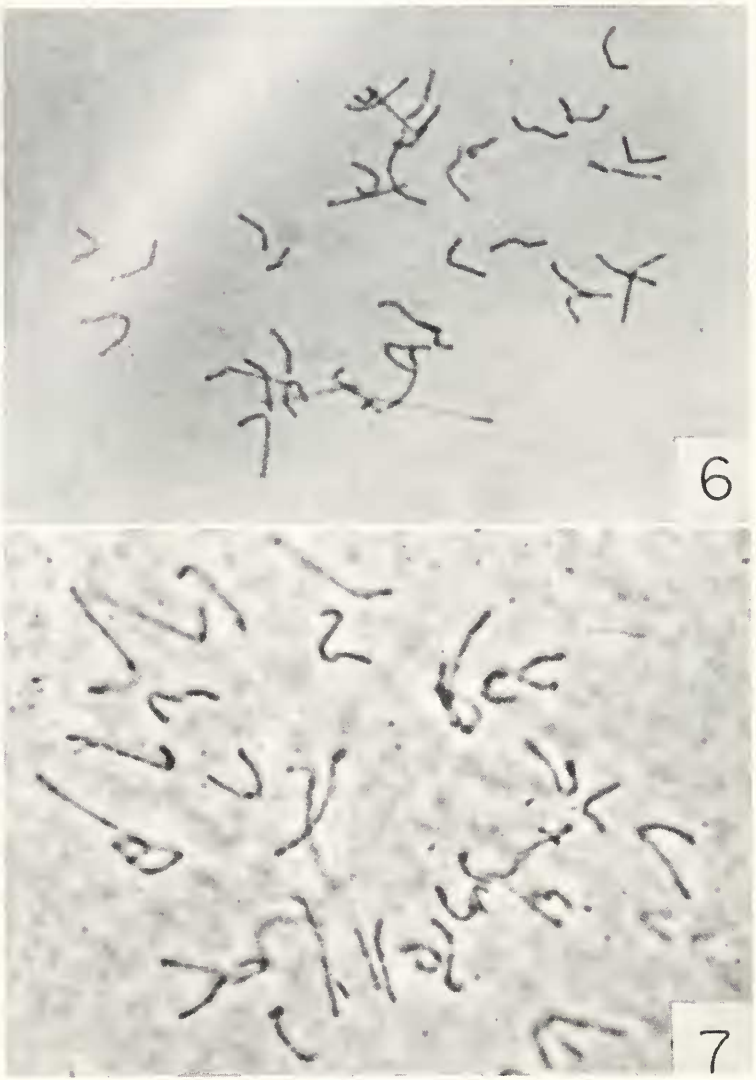


FIGURE 6. Metaphase of the second cleavage division in a fertilized egg of *Mercenaria campechiensis*, 38 chromosomes. Acetocarmine staining, × 1800.

FIGURE 7. Metaphase of normal first cleavage division, F_2 hybrid, 38 chromosomes. Acetocarmine staining and phase-contrast illumination, × 1800.

recognizable though degenerating metaphase I configurations were present 20 hours after spawning. If an effective sperm suspension was added, meiosis proceeded rapidly, the first polar body appearing in 10 minutes, and metaphase II in 15 minutes. From metaphase I through telophase II the sperm pronucleus was discerned as an increasingly diffuse nucleus lying at some distance from the meiotic spindles. Fusion of the egg and sperm pronuclei was not identified with certainty but probably occurred when the chromosomes of both were in a mid-prophase

condition preceding formation of the first cleavage spindle. The first cleavage division of the zygote nucleus ensued as early as 20 minutes and usually within 30 minutes after spawning. Subsequent cleavage divisions followed rapidly; eggs 75 minutes after spawning and contact with sperm suspension often contained too many dividing nuclei for analysis.

Systematic comparisons of the timing of development in the various types of fertilizations were not made. Preliminary observations suggested that fertilizations in which sperm from the F_1 hybrids were used (backcrosses to both parental species and F_2) were followed by somewhat delayed development. In one lot of F_2 embryos fixed on October 27, 1964, first cleavage metaphase and anaphase were found in lots fixed 45–75 minutes after sperm contact. Among 18 embryos in which chromosomes could be counted, 5 were diploid, 4 triploid (Fig. 16), 7 tetraploid, one had a chromosome number (46) between diploid and triploid, and one egg had a diploid and a separate haploid nucleus, both at metaphase. Subsequent lots of eggs from similar fertilizations did not exhibit polyploidy (Fig. 7). Occasionally an early embryo with dividing haploid nuclei was observed in batches of eggs which had not been fertilized either because sperm were not added or because the sperm were ineffective. A careful comparison of rates of development under controlled conditions should be made.

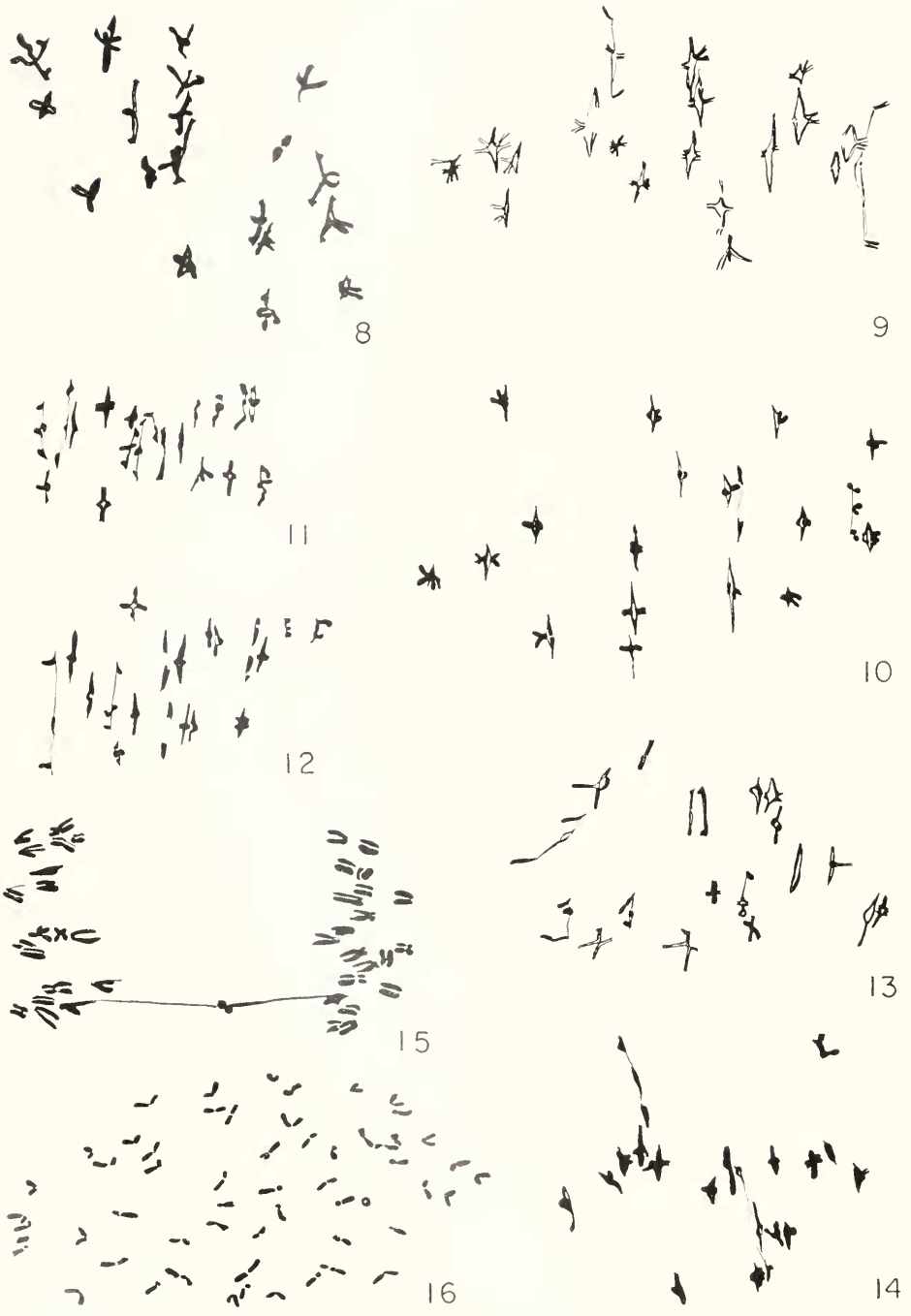
Chromosomes

Both *M. mercenaria* (Figs. 5, 11, 12) and *M. campechiensis* (Figs. 1–3, 8–10) have 19 pairs of chromosomes at metaphase I and 38 chromosomes at embryonic mitoses (Fig. 6). At metaphase I the chromosome pairs are small and slender and the chromatid split can often be discerned. Most of the chiasmata do not terminalize until the onset of anaphase. A typical nucleus from *M. campechiensis* (Fig. 9) showed 19 bivalents with 27 unterminalized and 8 nearly or completely terminalized chiasmata (1.89 chiasmata per chromosome pair). The metaphase I bivalents of *M. mercenaria* are similar, but in our material tended to be more compact and hence less easily analyzed for chiasma frequency and position. The F_1 hybrid was intermediate in this regard, some figures approaching those of *M. campechiensis* in clarity (Figs. 4, 13, 14).

In the F_1 hybrid all the chromosomes were paired regularly as 19 homomorphic bivalents at metaphase I. The chiasma frequency was not conspicuously different from those of the parents.

Later stages of meiosis proceeded conventionally in the two species and in the hybrid. In one batch of eggs from the hybrid, two anaphase I figures showed an apparent bridge between the two groups of chromosomes, one of which is shown in Figure 15. Since no fragments were found, the bridges probably resulted from lagging separation of chiasmata rather than from crossing-over within a heterozygous inversion. At anaphase I in both species and the hybrid the chromosomes at one spindle pole were commonly more compact and darkly stained than those at the other. In our squashes we were unable to tell whether either the darker or lighter group was consistently destined to be included in the first polar body.

The mitotic chromosomes of the first and second cleavage divisions were rather long and very slender (Figs. 6, 7) but tended to become shorter and more compact, at least at metaphase, in later divisions. Because of the rather high chromosome



FIGURES 8–16. Chromosomes in fertilized clam eggs. Drawings made with the aid of a camera lucida; all × 900 except Figure 15, × 1125.

number and small cells, it was not practicable to count the chromosomes of individual nuclei after the second cleavage metaphase. The individual chromosomes exhibited a rather wide range of relative lengths and of arm length ratios. Since the meiotic bivalents of the hybrid revealed no evidence of structural differences between the two species, detailed comparisons of mitotic karyotypes were not made.

Discussion

The ease with which hybrids between *M. mercenaria* and *M. campechiensis* can be made experimentally and the existence in nature of forms which can be interpreted as intermediate suggest that a certain amount of gene flow may occur between the two taxa. The homology and regular behavior of the chromosomes of the two species revealed at meiosis in the F_1 hybrid demonstrate that there is no gross chromosomal barrier to such gene interchange.

Ability to exchange genes under experimental conditions does not, of course, imply that such interchange actually does occur in nature. Mayr (1963) has recently reviewed the mechanisms which may serve to keep populations separate even though they are sympatric in part of their range and can be successfully hybridized under experimental conditions. Porter and Chestnut (1960) suggested that in the region of Beaufort, North Carolina, where *M. mercenaria* is confined to inland bays and inlets and *M. campechiensis* to outer shallow neritic waters, the two populations may be effectively separated by differential tolerance to salinity. The preliminary observations of F_2 and back-crosses suggest also that embryos of the species may have an advantage in rate of development over the hybrid offspring under certain conditions.

Regardless of whether and to what degree hybridization and gene exchange between *M. mercenaria* and *M. campechiensis* occur in nature, results so far suggest that their hybrids could furnish an important source of variation for the selection of improved strains of clams for commercial production, especially for regions in which the commercially less desirable *M. campechiensis* is naturally better adapted.

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Summary

Chromosome numbers of $n = 19$, $2n = 38$ are reported for *Mercenaria mercenaria, M. campechiensis* and their F_1 hybrids. Meiosis is normal in the hybrids

Figures 8–10. Meiotic metaphase I in *Mercenaria campechiensis*, 19 bivalents, mostly with one or two unterminalized chiasmata. Figure 8 is the same nucleus as Figure 1; Figure 10, the same as Figures 2 and 3.

Figures 11, 12. Meiotic metaphase I in *Mercenaria mercenaria*, 19 bivalents. Figure 12 is the same nucleus as Figure 5.

Figures 13, 14. Meiotic metaphase I in the F_1 hybrid, 19 bivalents, the chiasma frequency not conspicuously different from that of the parents.

Figure 15. Anaphase I in the F_1 hybrid showing one bridge or (more likely) pseudobridge.

Figure 16. Metaphase of an aberrant triploid first cleavage division, F_2 hybrid, 57 unusually short chromosomes.

and yields no evidence of chromosome nonhomology or structural rearrangements between the two parents. The hybrids produce functional eggs and sperm which result in normal fertilization and early embryonic divisions in reciprocal backcrosses and at least some F_2's. No gross chromosomal barrier to gene exchange appears to exist between the two species.

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